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EXPERIMENTS WITH FLAG SMUT OF WHEAT AND THE CAUSAL FUNGUS, UROCYSTIS TRITICI KCKE.[1]

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INTRODUCTION

Urocystis tritici Kcke., the causative organism of flag smut of wheat, though long known to be common in Australia and certain Asiatic countries, was not observed in the United States until May, 1919, when it was discovered by Dr. J. G. Dickson in a wheat field near Granite City, Ill. Because of its reported destructiveness to the wheat crops in other parts of the world, especially Australia, experiments in varietal resistance of wheats were begun in the fall of that year at Granite City. This line of investigation was considered the most expedient at that time because it was known that infestation of soil rather than of seed was the most important factor in the annual occurrence of the disease in Australia. From previous experiments in that country, it had been shown that seed treatment was ineffective in controlling flag smut in infested fields because of infection by viable spores in the soil. Thus, by selecting and sowing varieties which do not become infected in the field, even with artificial inoculation, the disease could be controlled. The results of these and later field experiments relating to varietal resistance and seed treatments have been published recently by Tisdale, Dungan, and Leighty (*15*).[3]

The data here presented are based on greenhouse experiments begun in the autumn of 1919 at Arlington Experiment Farm, Rosslyn, Va. In the first year only preliminary experiments were attempted, such as the testing of 25 varieties of wheat for susceptibility to flag smut and making a detailed study of the diseased plants. In the autumn of 1920 the number of varieties was increased, and as the work progressed and numerous problems presented themselves it was decided to study some of the physiologic aspects of the disease during the following years. The varietal resistance studies, therefore, cover a period of three years, while the other data are based mainly on a year's investigation conducted at Washington, D. C., and Rosslyn, Va., and a second year's study conducted cooperatively with the Missouri Botanical Garden, St. Louis, Mo.

[1] Received for publication Nov. 23, 1923.

[2] The writer is indebted to Dr. G. M. Reed, formerly pathologist in charge of cereal smut investigations, under whose direction the varietal resistance experiments were begun; to Dr. W. H. Tisdale, who succeeded Doctor Reed in 1920 and whose aid has been invaluable in the greater part of these investigations; and to Dr. B. M. Duggar, of the Missouri Botanical Garden, St. Louis, Mo., who gave helpful advice concerning the 1922–23 experiments reported in this paper.

Most of the seed used in these experiments was obtained from the agronomic division of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, though that of one or two varieties was obtained from each of the State agricultural experiment stations at Knoxville, Tenn., Urbana, Ill., Manhattan, Kans., Davis, Calif., and Pullman, Wash.

[3] Reference is made by number (italic) to "Literature cited", p. 448–449.

DISTRIBUTION AND ECONOMIC IMPORTANCE

To date (1923) flag smut of wheat has been reported as occurring in the United States, Australia, Japan, China, India, South Africa, Italy, and Spain. In the United States it has been found in Monroe, St. Clair, Madison, Jersey, Macoupin, Greene, Scott, Hancock, and Logan Counties in Illinois; in St. Louis, St. Charles, Warren, Platte, and Buchanan Counties in Missouri; and in Atchison, Leavenworth, Wyandotte, and Miami Counties in Kansas. Field surveys to determine the distribution of flag smut have been made by the State of Illinois and the United States Department of Agriculture each summer for the period from 1919 to 1923. The survey data for each year show that flag smut was found more widely distributed than in the previous year. This increase in the infested area may be due partly to the natural spread of the disease, and partly to the fact that larger areas were surveyed each year, or to either cause alone. From the earlier data it appeared that the disease was spreading rapidly, but evidence from the 1922 and 1923 surveys indicates that it probably has been present for two to several years even in areas reported for the first time to be infested.

During the five years that flag smut has been known to occur in the United States the annual loss due to this disease in the surveyed areas probably has been less than 2 per cent. In parts of some fields, however, 50 per cent of all plants within a given area have been infected. The low average percentage may be due partly to climatic conditions and partly to the fact that most of the time the infested areas have been under State quarantine regulations with regard to disinfection of all grain and threshing machines, the movement of straw, and the sowing of varieties which were found to be fairly resistant to flag smut.

In the literature from Australia, where flag smut was noted first in 1868 (*9*), losses of from 10 to 50 per cent of the wheat crop, due to this disease, commonly have been reported. However, according to information given by R. J. Noble, of Sydney, Australia, the average annual loss due to flag smut alone is about 3 per cent. In some years it is considerably higher, while in others it is practically negligible. Although flag smut has been distributed widely in the eastern wheat-growing area of Australia, it was not known to occur in Western Australia. However, W. M. Carne, who is engaged in plant research in Western Australia, in a letter to Dr. R. J. Haskell, stated that flag smut was recorded as occurring for the first time in that State in October, 1922, but on one farm only.

Butler (*2, p. 171–173, fig. 56*) states that flag smut is confined to the Punjab in India, and that "it has not been reported as causing much damage" in that country.

Hori (*5*) states that flag smut has been known in Japan since 1895 and that within an area of one-fourth acre, in the Province of Kai, in 1898, 100 per cent of the plants were destroyed before flowering time. It has been reported by Hori (*4*)[4] from Gumma and Yamanshi prefectures, and from Higo Province by Yoshino (*17*).[4] Flag smut of wheat is not known to occur in Formosa and Korea. Hori (*6*)[4] further states that this smut is not so well known to the farmers as the other grain smuts, though infrequently it causes considerable damage.

[4] Japanese original translated by Dr. T. Tanaka, formerly botanical assistant and translator, Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

Miyake (*10*) reports the occurrence of flag smut in China and states that it is "very common in Peking and its vicinity."

Putterill (*12*) reports the occurrence of flag smut in South Africa, and states that it probably has been present there for a "number of years," and that "while the total loss up to now may not be considered very great in that district [Marico], yet in some wheat fields lately visited almost half of the crop was found to be affected."

Flag smut of wheat was collected by E. C. Stakman in 1922 at Rieti, Italy, and at Zaragossa, Spain. The economic importance in either country was not determined.

SYMPTOMS OF THE DISEASE

The first indication that a plant in the field is infected with flag smut is the appearance of long grayish-black stripes running parallel with the veins on the upper leaves. At this stage diseased plants in the field appear to be as vigorous as the healthy ones. At a later stage the leaves and upper part of the culms become twisted and curled. Upon close examination of infected plants grown in the greenhouse and field it was found that the young upper leaves showed stripes which were white at their base, becoming whitish gray and finally a lead-gray in color toward the apex of the leaf. Later the epidermis of the leaf ruptures along these stripes and exposes the spores of the fungus in black sooty masses. Plate 1, A, shows some infected leaves with the typical sori. An enlarged portion of an infected leaf also is illustrated (Pl. 1, B). The stripes, or sori, generally are confined to the leaves, although they may occur frequently on the leaf sheaths (Pl. 1, C), occasionally on the upper part of the culm, and still more rarely on the lower glumes and rachis of the wheat head (Pl. 1, D). Plate 1, C, shows the infection occurring on the lower glumes of an underdeveloped head. In many cases all the culms of a diseased plant are infected, although an occasional plant in the greenhouse or field may be found with but one infected culm and the others apparently free from the disease and producing well-filled heads.

In general, the disease seems to stunt the growth of the culms so that no heads are produced. However, in the field in 1922 and 1923 cases were noted where the smutted culms apparently were producing normal heads. These, upon examination, were found in many cases to be sterile, though occasionally a few seeds in each head matured. McAlpine (*9*) has noted cases where small amounts of shriveled grain were produced from infected culms. In a few of these individuals producing sterile heads it was noticed that the infection was confined entirely to the upper part of the culms, there being no infection of any of the leaves of that culm or of the remainder of the plant.

In the greenhouse, plants of some varieties of spring wheat become infected before they begin to tiller. Very susceptible winter varieties also become infected before or at about the time they begin to tiller. Infection may appear at any time from these young stages of development until the plants are mature.

The culms of infected plants grown in the greenhouse do not tend to curl and twist, as commonly is the case in the field. Infected plants were not allowed to mature, which fact might account for the difference in the effect on plants grown in the greenhouse and on those in the field. It was noticed, however, that in certain varieties, such as Hard Federa-

tion, White Federation, and Bobs, of spring habit, the culms often show this character whether inoculated or not. Obviously, then, this can not be regarded as a trustworthy indication of the disease in these particular cases.

During the first year a detailed record was kept for each infected plant of the different varieties. Each plant was examined separately and the number of well-developed culms determined. In addition, the small undeveloped culms were noted. The nodes from which the roots developed, or the crown, were regarded as No. 1, and the others were numbered successively from the base to the apex. The leaves from the first node usually were dead and dry at the time the plants were examined. This also frequently was true of the leaf at the second node, and sometimes also of that at the third, depending upon the maturity of the plant. However, even in this semidry condition, it was possible to determine whether or not these lower leaves were infected. These plants had reached the heading stage, except in a few varieties where the severity of infection necessitated their removal from the greenhouse before that time.

A summary of the data concerning total culm infection for nine varieties in the crop year 1919–20 is given in Table I.

TABLE I.—*Summarized results showing culm infection of wheat plants of different varieties infected with Urocystis tritici and grown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1919–20*

Variety.	Source or C. I. No.	Number of plants—		Percentage of plants with all culms infected.	Culms of partially infected plants.		
		With one to all culms infected.	With all culms infected.		Total number.	Number infected.	Per cent infected.
Baart	1697	16	0	0	113	43	38.1
Bobs	4990	40	24	60.0	122	93	76.2
Cowra No. 3	4119	38	30	78.9	52	37	71.2
Defiance	Calif.	38	5	13.2	268	146	54.5
Fultz	3598	18	0	0	217	114	52.5
Hard Federation	4733	37	29	78.4	51	37	72.5
Harvest Queen	5957	41	28	68.3	142	110	77.5
Do	Ill.	31	17	54.8	143	116	81.1
Propo	1970	18	2	11.1	96	31	32.3
White Federation	4981	40	29	72.5	45	29	64.4

In no variety were all culms infected on all infected plants, though in five varieties over 60 per cent of the plants had all their culms infected, while in two varieties no infected plant had all its culms infected. Of the partially infected plants—namely, those which had both sound and infected culms on the same plant—the percentage of culms infected ranged from 32.3 per cent to 81.1 per cent. In seven of these varieties over 50 per cent of the culms were infected.

In general, every well-developed culm of an infected plant showed infection, the severity being indicated by the number, length, and size of the sori. Rarely a leaf showed no infection, though sori appeared on the leaves above and below. As a rule, however, any culm with the second or third leaf infected would show sori on all other leaves above.

Over 1,600 culms were examined to determine the frequency of the occurrence of sori on the lower leaves of the culms. Sori were found on all the leaves on 16.7 per cent of the infected culms; on the second leaf and all leaves above on 48.7 per cent of the infected culms; on the third leaf and all above on 25.3 per cent; on the fourth and those above on 6.7 per cent; on the fifth and those above on 2.4 per cent; and on the sixth and any above on 0.2 per cent. When infection was noted on the first and second leaves there usually was just a trace of the disease, the infection increasing in severity from the third leaf on up to the apical leaf.

The diseased leaves generally were shorter and narrower than the corresponding leaves of healthy culms. The leaves of sound and of infected culms from the same infected plants of 12 varieties were measured and, even in these cases, the diseased leaves were consistently shorter and narrower than the corresponding leaves on the sound culms of the same plant.

CAUSAL ORGANISM

Flag smut of wheat is caused by a fungus, *Urocystis tritici* Kcke., belonging to the family Tilletiaceae Tul. It was reported by McAlpine (*9*) as occurring in Australia as early as 1868 and determined by Wolff (*16*) in 1873 to be *U. occulta* (Wall.) Rab., the species which occurs on rye (*Secale cereale*). In 1877 Körnicke (7), on examining specimens of flag smut from Australia, separated it from *U. occulta* upon morphological characters alone and named the new species *U. tritici*. However, the name was not adopted, and Saccardo (*13, p. 515*) gave this name as a possible synonym for *U. occulta*, and Sydow and Butler (*14, p. 427*), McAlpine (*8*), and Hori (*5*) still reported this fungus as *U. occulta*.

McAlpine (*9*) conducted cross-inoculation experiments with Urocystis from wheat and rye in 1907 and 1908. He concluded that "the Flag smut of wheat and rye are not mutually infective, and therefore the name given to Flag smut of wheat by Koernicke in 1877, who received specimens from R. Schomburgk in South Australia, should be retained, viz., *Urocystis tritici*."

Urocystis tritici is not known to infect plants of any genus other than Triticum, though several genera have been found to be infected with other species of Urocystis. In 1921, plants of redtop were received which were infected with Urocystis. During the winter of 1921–22 two lots of seed of redtop, Pal timothy, St. John rye (C. I. 130), and Little Club wheat (C. I. 4066) were inoculated, one lot with *U. tritici* and the other with *U. occulta*. These lots of seed were sown on December 1, 1921, in the greenhouse at Rosslyn, Va. There was insufficient spore material from the infected redtop plants to inoculate similar lots of seed. *U. tritici* infected all, or 100 per cent, of the wheat plants, but did not infect any of the rye, timothy, or redtop plants. *U. occulta* infected 7.1 per cent of the rye plants, but did not infect any of the wheat, timothy, or redtop plants.

The spores of flag smut are either solitary or held together in groups of two to several, and the whole completely invested by a layer of small sterile cells (Pl. 2, A). Each spore of a spore ball may germinate, though usually only one or two actually do so (Pl. 2, E, G). However, spore balls with three and even four germinating spores are frequently noted (Pl. 2, H). On germination, the spore sends out a promycelium (Pl. 2, B) with a whorl of one to several sporidia at the apex (Pl. 2, C, D). These sporidia do not become separated from the

promycelium but elongate to form the so-called infection threads (Pl. 2, F *a*, H *d*). Frequently not all of the sporidia elongate (Pl. 2, F *b*), but eventually appear to lose their protoplasm and become hyaline. Fusion of these elongated sporidia and also the production of secondary sporidia (Pl. 2, I *a*) were observed in a very few cases, though in the great majority of instances they merely became elongated. The size and length of the promycelia, sporidia, and infection threads varied with the conditions of germination.

SPORE GERMINATION

The spores of *Urocystis tritici* do not germinate readily or consistently in either distilled or tap water. With spores from material about a month old and kept seven days on soil, McAlpine (*9*) secured limited germination in a water culture after 24 hours incubation. Approximately 40 per cent of the spores of the previous season germinated after being floated on tap water for about four days.

Various solutions and media were tried under different conditions of moisture and temperature by the writer, but though the spores free from any leaf tissue would germinate under one set of conditions at one time, they would not germinate under apparently similar conditions at another. Thus many negative results were obtained. It may be of interest to note those conditions under which fairly high percentages of germination were secured. In 1920 a method was found which since then has given fairly high and uniform results. In these studies spore-laden leaves were powdered and used as a fine dust-like mixture instead of using spores free from leaf tissue. This material was floated on distilled water in a Syracuse watch glass and placed at 18° to 20° C. Some spores germinated at the end of two and three days, although the best germination usually was had on the fourth day. The germination, once started, proceeded very rapidly, and the elongated sporidia, or infection threads, were quite numerous on the fifth day.

Good germination was secured many times in 1921 in tap-water cultures after two to five days incubation. The spores free from leaf tissue, however, did not germinate readily in distilled water, though a few spores sometimes germinated after four or five days. The optimum temperature for germination was 18° to 20° C., though germination at 25° was recorded in one culture and in certain others at 12° to 13°. Satisfactory germination was obtained at 10° after seven to eight days when spores mixed with pulverized leaf tissue were used. However, in infection experiments herein described no infection was obtained at 25°, though it did occur at 6° to 12°.

In December, 1921, fairly good germination took place after three to four days in juice expressed from wheat seedlings and diluted. In some instances germinating seeds were found to stimulate spore germination. In October, 1921, spores which had been dusted on seeds which were placed on soil in a Petri dish in the laboratory germinated freely after two days, while spores from the same source dusted on soil and on the surface of both tap water and a soil solution, did not germinate after four days of incubation.

During the course of these experiments this stimulating effect of germinating seeds on spore germination was observed also in some tests of the viability of spores buried in the soil. For these experiments 14 samples of spores which had been buried under different conditions were

used. The soil and spore mixture constituting each sample was divided into duplicate portions, each of which was placed in a Syracuse watch glass and 3 cc. of tap water added to each. In one portion of each sample five wheat seeds were placed. After four days there was good spore germination in four of these portions in which seeds were germinating, but there was no germination in the duplicate portions without seeds, with the exception of one in which a single germinating spore was found. Also there were a few spores germinating in one other dish in which no seeds had been placed. After seven days there was good germination in four more cultures containing seeds, but there was no increase in germination in the lots without seeds.

Brown (*1*) recorded the stimulating effect of volatile substance arising from plant tissues on the germination of spores of *Botrytis cinerea* and other fungi. Plant distillates and various chemical substances also stimulated germination. Noble's data (*11*) confirm Brown's results on the effect of plant tissues and distillates on spore germination. He found that spores of *Urocystis tritici* "which had been presoaked in water for several days would germinate profusely after the addition of small quantities of wheat seedlings tissue."

VIABILITY OF SPORES

In order to test the viability of spores of various ages, seeds of Harvest Queen (C. I. 5957) were inoculated with dry spores of *Urocystis tritici*, collected in different years and at various places, as given in Table II. The inoculations were made by mixing the seed with the spores in small seed envelopes. The inoculum used in this experiment was obtained from leaves which had been dried and kept at ordinary room temperature under laboratory conditions. The inoculated seed was sown in the greenhouse and infection noted on the plants as they matured. The infection percentages are recorded in Table II.

TABLE II.—*The effect of age of spores of Urocystis tritici, and the locality in which they were produced, on the power to infect Harvest Queen wheat in the greenhouse at Arlington Experiment Farm, in 1921–22*

Row number.	Source of spores.	Year collected.	Wheat plants.		
			Total number.	Number infected.	Per cent infected.
37	Australia [a]	1919	16	16	100
55	Granite City, Ill.	1919	14	9	64.3
46	Rosslyn, Va.	1920	17	9	52.9
64,73	Do.	1921	31	21	67.7
10,19,28	Granite City, Ill.	1921	49	23	46.9

[a] Spores obtained in 1921 from R. J. Noble.

TABLE II shows that the spores produced in 1919 in Australia caused 100 per cent infection, while the others were from 46.9 to 67.7 per cent effective. It may be that the fungus from Australia is more virulent than that obtained in the United States. However, these differences may not be significant, as was shown by the following experiment. Seed of Harvest Queen from the same source as that used in the previous ex-

periment was inoculated with spores collected in the greenhouse at Rosslyn, Va., in 1921, and sown in the same greenhouse bench about 18 days later than the sowings recorded in Table II. Fourteen out of 15 of these plants, or 93.3 per cent, became infected. The seed and spores were from the same source as those used in rows 64 and 73, given in Table II, in which but 67.7 per cent of the plants became infected. Therefore it is reasonable to expect that had the conditions at the time of sowing been similarly varied for all the spore lots used in the inoculations noted in Table II, different percentages of infection might have been obtained. However, the results serve to indicate that the infective power of spores is not destroyed even though they are stored two or three years under laboratory conditions.

In the spring of 1923 germination tests were made with spores from the same collections listed in Table II. In all instances good germination was obtained. Thus it has been shown that the spores collected in Australia and Illinois in 1919 were viable after having been kept for four years in the laboratory.

In order to test the infective power of fresh spores, seed of Harvest Queen was inoculated with spores from infected green leaves collected on January 5, 1922, and sown in clean soil on January 6, 1922, together with one uninoculated row and one row inoculated with dry spores of the previous season for controls. The fresh spores produced an infection of 5.7 per cent and the dry spores 47 per cent, while no infection developed in the uninoculated row. Again in March, 1923, 30 seeds each of Harvest Queen, Hard Federation, and Little Club were inoculated with spores which had been collected the same day, and then were sown in three 10-inch pots containing garden soil autoclaved for 15 minutes at 15 pounds pressure. The pots were set in the soil on a greenhouse bench in order to lessen drying out and to prevent rapid temperature changes. Two plants of Hard Federation, or 6.7 per cent, became infected, but no infection developed in Harvest Queen or Little Club. Thus it was shown that wheat plants can become infected by fresh spores, though the percentages of such infection may be quite small.

OVERWINTERING OF SPORES

It having been shown that soil infestation was an important factor in the dissemination of flag smut, and one which made seed treatment partially ineffective, it was thought desirable to secure experimental data on the overwintering of the spores in the soil. On such information recommendations could be based for such practices in soil management as might control the disease.

The following experiment was conducted to determine the ability of spores to overwinter in the soil at Granite City, Ill., in the flag-smut area. Forty-two small cylindrical wire baskets about 1 inch in diameter and 3 inches long were filled with the various mixtures of different soils, diseased leaves, and spores of the previous season, as given in Table III. Besides the native black alluvium of Granite City, there were used sandy loam and basaltic soils from Madison, Wis., and Pullman, Wash., respectively. These baskets were buried in the ground at different depths on October 6, 1921.

One-third of the baskets were dug up on November 1, one-third on November 28, and the final third on April 10, 1922, and the contents sent to the laboratory in small glass vials. A soil suspension was made from each, using about 30 cc. of tap water. The spores in part were

recovered from the soil by fractional centrifuging. The heavier soil particles with many spores were centrifuged out first and then a layer, consisting of many spores and the lighter soil particles, was obtained. To test spore germination, this layer was pipetted off into a Syracuse watch glass, tap water added, and the whole placed in an incubator at 18° C. But it had been found that flag-smut spores often fail to germinate in cultures, although spores from the same source produced abundant infection in susceptible plants inoculated with them. So it was decided to grow seedlings in the heavier residue to determine the viability of these overwintered spores by their infective power. Five seeds of wheat were sown in each soil and spore residuum and allowed to grow in the same chamber with the spore germination tests. Harvest Queen was sown in the series dug up on November 1 and November 28, while in the series dug on April 10, 1922, both Harvest Queen and Bobs were sown.

When these seeds had germinated and each plant had one well-developed leaf, they were transplanted to clean soil in the greenhouse. Infection in the earlier sowings was first observed in January, and the final results were recorded in June. These data, together with the results of the spore germination tests, are recorded in Table III.

TABLE III.—*Viability and infective power of spores of Urocystis tritici buried for varying periods in different types of soil at Granite City, Ill., on October 6, 1921*

Soil and inoculum.	Depth of burial.	Dates baskets were dug from soil.						
		Nov. 1, 1921 (Series 1).		Nov. 28, 1921 (Series 2).		Apr. 10, 1922 (Series 3).		
		Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.	Infection on Bobs.
	Inches.		*Per ct.*		*Per ct.*		*Per ct.*	*Per ct.*
Black alluvial soil plus spores.	0– 2	—	40.0	+	0	—	0	0
Do.	2– 4	—	0	+	0	—	0	0
Do.	4– 6	—	75.0	—	0	—	0	0
Do.	6– 8	—	75.0	—	0	—	0	0
Do.	8–10	—	50.0	—	0	—	0	0
Black alluvial soil plus infected leaves.	0– 2	+	0	+	0	—	0	0
Do.	2– 4	—	0	+	0	—	0	20
Do.	4– 6	+	40.0	+	0	—	0	0
Do.	6– 8	+	0	—	0	—	0	20
Do.	8–10	—	0	+	0	+	0	0
Basaltic soil plus spores.	4– 5	—	33.3	—	0	—	0	0
Basaltic soil plus leaves.	4– 5	—	40.0	+	20	—	0	0
Sandy loam plus spores.	4– 5	+	30.0	—	0	—	0	0
Sandy loam plus leaves.	4– 5	—	0	—	0	—	0	0

The foregoing results seem to indicate that the infective power of the spores was markedly decreased during the time between the removal of the first and second series, or between November 1 and 28. The spore germinations show a slight increase, due probably to a difference in the method of germination. The two cases of infection obtained in Series 3 from spores buried over five months were noted on Bobs, a very susceptible spring wheat which had not been used for the earlier tests.

Another series of experiments on the overwintering of spores was conducted cooperatively with the Missouri Botanical Garden during the winter of 1922–23. Diseased leaves had been collected in 1922 from plants grown in the field at Granite City, Ill., and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. These leaves were ground in a food chopper, mixed with two parts of soil in a Mason jar, and placed in small, 2-inch flowerpots on November 24, 1922. These pots were divided into two lots and buried in the soil so that the tops of one lot were 1 inch below the surface of the ground and those of the other 5 inches below. The height of the pots being 2.5 inches, the spore mixtures in each pot of the first lot were from 1 to 3.5 inches and those in the other from 5 to 7.5 inches beneath the soil surface. The two depths at which the pots were buried are recorded in Table IV as 2 and 6 inches, respectively. In addition, six pots, similarly filled, were watered and kept in the greenhouse for 4, 8, and 12 days. At the end of each of these three periods, two pots were buried, one at each depth recorded above. As the viability of the spores is considered to be decreased during long intervals of wetness, the late sowings of spores which had been watered for 4, 8, and 12 days before burial were intended to simulate to a slight extent the periods of fall rains. Thus, if the spores had been in the soil through late summer and early fall, their viability might have been lessened by the time these experiments were begun. As a check on the viability of the spores a part of the soil and spore mixture was kept in the Mason jar until the first pots were dug on January 4, 1923.

At various intervals of about two weeks, some of the pots in each series were taken into the greenhouse. Seed of Little Club was sown in the pots and allowed to grow. The soil temperatures for the different sowings were not the same, the temperatures for March and April being considerably higher than those for January and February. After the resulting seedlings had produced one or two strong leaves, they were transplanted in clean soil in the benches. The pot buried on November 24, with the spores 2 inches below the surface, had its contents destroyed by mice after it was dug up on January 4 and wheat seed sown. Also the seed sown on January 4 in the pot buried 6 inches below the soil surface on December 6 and brought in on January 4 was destroyed by mice. Additional seed was resown in this pot on January 12, 1923. The infection results are given in Table IV.

From Table IV it seems that watering the spores and keeping them in the greenhouse for 4, 8, and 12 days before they were buried in the soil did not diminish their viability. Indeed, four of the rows showed 100 per cent infection, which was slightly higher than that of the control. The spores showed a marked decrease in viability about February 3. The coldest weather of the year was during the two weeks following this time, the minimum temperature recorded being about 1° F. By February 18, the ground had begun to thaw and the pot at the 2-inch depth was brought in, though the one at the 6-inch depth could not be dug from the ground until February 22. There was an infection of 11.1 per cent in the plants grown in the pot buried at the 6-inch depth and brought in on March 16. There was no infection in the plants sown in pots removed from the soil on April 14, but the plants did not grow well because of the high air and soil temperatures in the greenhouse. However, as previously stated, there was infection in the plants sown in April, 1922, in the infested soil which had been removed from the field at Granite City, Ill., on April 10, 1922.

TABLE IV.—*Infections of Little Club wheat produced by spores of Urocystis tritici buried at different depths and for varying periods during the winter of 1922–23 at the Missouri Botanical Garden, St. Louis, Mo.*[a]

Average depth spores were buried.	Date when spores were—		Total number of plants.	Infected plants.	
	Buried.	Exhumed.		Number.	Per cent.
Inches.					
Control	.....	.....	22	21	95.5
2	Nov. 28	Jan. 4	28	25	89.3
6	....do.....	....do.....	25	25	100
2	Dec. 2	....do.....	19	19	100
6	....do.....	....do.....	27	27	100
2	Dec. 6	....do.....	8	8	100
6	....do.....	....do.....	24	20	83.3
6	Nov. 24	....do.....	25	24	96.0
2	....do.....	Feb. 3	24	6	25.0
6	....do.....	....do.....	23	12	52.2
2	....do.....	Feb. 18	26	5	19.2
6	....do.....	Feb. 22	22	6	27.3
2	....do.....	Mar. 1	18	3	16.7
6	....do.....	....do.....	20	0	0
2	....do.....	Mar. 16	18	0	0
6	....do.....	....do.....	18	2	11.1
2	....do.....	Apr. 14	18	0	0
6	....do.....	....do.....	18	0	0

[a] Experiments in cooperation with the Missouri Botanical Garden.

On October 5, 1921, a plot of land about 10 feet square, near the experimental field at Granite City, Ill., was inoculated thoroughly with flag-smut spores by spading under heavily infected wheat straw. On October 12, 1922, over one year later, clean seed of Harvest Queen wheat was sown in this plot. An uninoculated control plot of equal size, located some distance away, also was sown with uninfested seed of the same variety. In May, 1923, there were nine infected plants, or 2.4 per cent, in a total of 377 in the inoculated plot. None of the 370 plants in the control plot was infected.

Thus it has been shown that, though there is a decrease in viability, the spores are able to survive a winter and subsequent summer in the flag-smut area near St. Louis, Mo. They not only are capable of germination, but actually can infect wheat plants.

INFECTION

Regarding the infection of wheat by *Urocystis tritici*, McAlpine (9) states: "Infection occurs in the seedling stage, also when the young shoots are being formed, but not when the plant is above ground, . . ." He proved that infection did occur in the seedling stage, but based his conclusions concerning the infection of new shoots as they are formed during tillering, on some data given by Hecke (3). The latter cut back to the collar shoots of sound plants of the perennial rye (*Secale montanum*) in the autumn. He then dusted spores of *Urocystis occulta* on the exposed collar and covered the plant with manure containing the same kind of spores. In the spring the shoots produced were infected. Discussing Hecke's data, McAlpine says: "But a fresh light has been thrown on the infection of rye, and the same probably applies to wheat. By the experiments of Hecke . . . it was there shown that the spore has not only

the one chance of infecting the primary or terminal bud, but also the numerous chances of infecting the lateral buds produced beneath the surface of the soil and growing out into fresh stalks. There is not only seedling infection, but shoot infection, and it is decidedly to the advantage of the parasite to multiply the points of attack as much as possible."

If it be true that new shoots as well as the plumule are liable to infection before emerging from the soil, then factors which would hasten growth through the soil would shorten the infection period and, therefore, would lessen the chance of infection. Soil moisture and temperature would play an important part by influencing the germination both of the wheat seed and of the smut spores.

Experiments were conducted to determine the relation, if any, between infection and date of sowing, temperature, stage of growth prior to inoculation, and tillering. These experiments are discussed separately. In addition, experiments were conducted to determine the effects of cutting back inoculated plants at different periods of growth on the appearance of flag-smut infection.

DATE OF SOWING AS RELATED TO INFECTION

The following experiment was conducted cooperatively with the Missouri Botanical Garden, to determine if any relation existed between the date of sowing inoculated seed and the amount of infection produced. Harvest Queen was used throughout this experiment. Eleven sets of 125 selected seeds each were inoculated and sown in rod rows at three-day or four-day intervals from October 17 to November 21, 1922. The rows were 1.5 feet apart, and the seeds were spaced at intervals of a little less than 2 inches. All inoculations were made simultaneously with dry, ground spore material of the previous season. The vials of inoculated seed for the later sowings were kept in a cool, dry place in order to maintain the viability of both the spores and seed. Infection was first observed on April 28, 1923, on some of the plants of the first sowings. The dates of the various sowings are given in Table V, together with the total number of plants, number of infected plants, and the percentage of infection for each date of sowing.

TABLE V.—*Percentages of infection of Harvest Queen wheat by Urocystis tritici, as influenced by date of seeding from October 17 to November 21, 1922, in the Missouri Botanical Garden, St. Louis, Mo.*[a]

Date sown.	Plants.		
	Total number.	Number infected.	Per cent infected.
Oct. 17	31	19	61.3
Oct. 20	48	24	50.0
Oct. 24	37	14	37.8
Oct. 27	26	6	23.1
Oct. 31	24	5	20.8
Nov. 3	24	7	29.2
Nov. 7	50	7	14.0
Nov. 10	79	19	24.1
Nov. 14	73	0	0
Nov. 17	72	0	0
Nov. 21	69	0	0

[a] Experiments in cooperation with the Missouri Botanical Garden.

The percentage of infection decreased from 61.3 per cent in the sowing of October 17 to 20.8 per cent in the sowing of October 31. There was no infection in the rows sown on or after November 14. These variations in the percentage of infection were due probably to soil temperature and moisture conditions, which inhibited the germination of the spores at the time the wheat was germinating.

TEMPERATURE AS RELATED TO INFECTION

A series of experiments was arranged in order to determine the range of temperature within which actual infection could occur. On December 1, 1921, 40 seeds of each of five susceptible varieties of wheat (Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation) were inoculated heavily with flag-smut spores by moistening the seeds and rolling them in dry spores. The seeds were then incubated between moist blotting papers in the manner prescribed for standard seed-germination tests. Through the kindness of W. L. Goss, of the Seed Testing Laboratory of the Bureau of Plant Industry, germination chambers were secured in which the temperature was controlled within 2 degrees by electric thermostats and in which a humidity of practically 100 per cent was maintained. Recording maximum and minimum thermometers were placed in each chamber. The three chambers used had average temperatures of 17.8° C., 22.3°, and 25.8°, with fluctuations between 17° and 19°, 21.5° and 23.5°, and 25° and 26.6°, respectively, during the time the seeds were in the chambers. A fourth lot of seeds of the same varieties was placed in an ice box, the temperature of which ranged from 6° to 12° during the 30 days of the experiment.

When the seedlings in each chamber had reached the stage where the first leaf had pushed through the coleoptile, they were washed thoroughly with the aid of a brush until no spores were visible on the seeds. They were then transplanted to clean soil in the greenhouse, in December, 1921, and allowed to grow until flag-smut lesions appeared or, if the plants did not become infected, until maturity. The infection percentages obtained at each temperature are given in Table VI.

TABLE VI.—*The effect of temperature on infection of five varieties of wheat by Urocystis tritici when inoculated seed was germinated on blotters in temperature chambers, then washed and transplanted in greenhouse soil in December, 1921*

Variety.	Average temperature.											
	9° C.			17.8° C.			22.3° C.			25.8° C.		
	Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.	
		No.	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*
Bobs	30	5	16.7	25	3	12.0	19	6	31.6	27	0	0
Hard Federation	29	0	0	19	0	0	16	7	43.8	26	0	0
Harvest Queen	23	0	0	32	6	18.8	28	9	32.1	30	0	0
Little Club	23	0	0	23	1	4.3	27	6	22.2	31	0	0
White Federation	.....	....	....	18	0	0	14	2	14.3	14	0	0
All varieties	105	5	4.8	117	10	8.5	104	30	28.8	128	0	0

The data show that only one variety, Bobs, became infected at 6° to 12° C. and that the highest percentage infection for each variety occurred at 21.5° to 23.5°. The fact that no infection developed in any of the five varieties when grown at 25° to 26.6° is probably due either to the accelerated growth of the wheat plants at this higher temperature, which enabled them to escape infection, or to the inhibition of spore germination, or to both effects.

Even at the optimum temperature all of the percentages of infection are relatively low for such susceptible varieties. In the varietal resistance experiments for 1921–22, herein described, in which the inoculated seed was sown in the soil, the percentages of infection in these same varieties were 86.7 for Bobs, 80.8 for Hard Federation, 88.5 for Harvest Queen, 97.8 for Little Club, and 97.4 for White Federation.

A preliminary experiment on the effect of controlled soil temperatures on infection was conducted by W. H. Tisdale and R. W. Leukel in the greenhouse at Arlington Experiment Farm in the spring of 1923. An equal amount of soil was placed in each of 12 galvanized cans. Two cans were set in water in each of six tanks held at 10°, 15°, 20°, 24°, 28°, and 32° C., respectively. Twenty seeds of Bobs wheat were sown in each can on March 26. The seeds were spaced at regular intervals in a circle, 1.5 inches from the border of the can, and covered with about 1 inch of soil. No smut developed in any of the cans except the ones in which the soil temperature was held at 20°. In these cans 5, or 12.5 per cent, of the 40 plants became infected with flag smut.

STAGE OF GROWTH AS RELATED TO INFECTION

In the case of seedling infection it is stated by McAlpine (9) that infection occurs before the plant emerges from the soil. Soon after emergence the coleoptile, or sheath, which completely invests the plumule, is broken, and the latter appears. In order to determine the stages of seedling growth at which infection could occur, the following experiments were conducted. Seed of Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation, the same varieties used in the temperature experiments, were placed between moist blotters, at 18° to 20° C. in a seed-germination chamber on December 6, 1921. When these seeds were at three definite stages of germination—namely, (1) testa, or seed coat, just broken; (2) plumule about 1 to 1.5 cm. long, and three strong roots developed; and (3) coleoptile just broken, those in each stage were divided into two lots. In addition, dry, ungerminated seed was divided into two lots. Both lots were inoculated and placed in clean soil in the greenhouse December 9 to 16, 1921. One lot of each was inoculated by rolling the seed or seedling in dry spores and the other by immersing in a spore suspension. This suspension consisted of spores soaked in tap water for at least two days. The spores in suspension were not necessarily germinating. It was thought that if the time between the transplanting and emergence of the seedlings was very short the dry spores might not have time to germinate and infect the seedlings before the latter came through the ground. On the other hand, if the spores were thoroughly soaked they could germinate in a very short time. Unfortunately, the emergence dates for these seedlings were not recorded, although these data would have been valuable in correlating rate of growth and infection. Table VII contains the results for the two lots of each variety inoculated with the soaked and dry spores, respectively, at the three stages of growth described above.

TABLE VII.—*Results of inoculating wheat seedlings at three definite stages of growth with both soaked and dry spores of Urocystis tritici and planting them in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., December 9 to 16, 1921*

Variety.	Seedlings inoculated with—						Average percentage of infection including both lots.
	Lot 1. Soaked spores.			Lot 2. Dry spores.			
	Total plants.	Infected plants.		Total plants.	Infected plants.		
		Number.	*Per cent.*		*Number.*	*Per cent.*	
SEED COAT JUST BROKEN.							
Bobs	36	4	11.1	35	17	48.6	29.6
Hard Federation	34	5	14.7	33	16	48.5	31.3
Harvest Queen	32	6	18.8	34	6	17.6	18.2
Little Club	31	20	64.5	28	10	35.7	50.8
White Federation	33	20	60.6	30	23	76.7	68.3
PLUMULE AND THREE ROOTS DEVELOPED.							
Bobs	35	5	14.3	34	12	35.3	24.6
Hard Federation	35	9	25.7	36	11	30.6	28.2
Harvest Queen	34	10	29.4	33	4	12.1	20.9
Little Club	30	25	83.3	31	18	58.1	70.5
White Federation	29	18	62.1	29	16	55.2	58.6
COLEOPTILE BROKEN.							
Bobs	35	0	0	36	0	0	0
Hard Federation	36	0	0	36	0	0	0
Harvest Queen	35	0	0	34	0	0	0
Little Club	32	0	0	33	0	0	0
White Federation	.....	.....	.....	.....	.....	.....	.....
CONTROL: DRY UNGERMINATED SEED.							
Bobs	17	8	47.1	.....	.....	.....	47.1
Hard Federation	17	8	47.1	.....	.....	.....	47.1
Harvest Queen	15	7	46.7	17	9	52.9	50.0
Little Club	8	8	100	8	6	75.0	87.5
White Federation	7	5	71.4	7	5	71.4	71.4

Abundant infection occurred in the plants inoculated at the first two stages, but in no case was there infection of any plant which was inoculated after the coleoptile was broken. The highest percentage of infection in the three-root stage was 83.3 per cent in Little Club, and in the stage where the seed coat was just broken there was 76.7 per cent in White Federation. There apparently was no relation between the condition of the inoculum at time of application and the percentage of infection produced in each case. In some instances where the inoculations were made at the same stage of growth the dry inoculum produced a higher percentage of infection, while in others the spore suspension produced the higher percentage. Four of five varieties had higher percentages of infection when inoculated with dry spores at the time the seed coat was just broken than when the inoculations were made at the three-root stage. All of the varieties inoculated with the spore suspension had higher percentages of infection when they were inoculated at the three-root stage than when the seed coat was just broken. In the varietal-resistance experiments herein described infections of 100 per cent were

recorded in a few varieties. Obviously, in these cases, inoculating the seeds with dry spores gave as high a percentage as could have been obtained if the seeds had been inoculated with soaked or even germinating spores. These results would indicate that the period favorable for infection is before the seedling comes through the ground, or before the coleoptile is broken.

In order to test the possibility of infection during the tillering stage, when new shoots are produced from the crown of the plant, the following experiments were conducted: One lot of smut-free seed of Harvest Queen, Little Club, and White Federation, the same varieties used in the foregoing experiments, was sown in clean soil on November 28, 1921. A similar lot was inoculated with dry spores of the previous season and sown on the same day. Three rows of each variety in the two lots were sown. On December 13, when the plants in both lots had developed one strong leaf and a small second one, they were removed from the soil. Only two rows of each variety in the two lots were removed. For a control the third row in each instance was not disturbed. The uninoculated plants then were inoculated by rolling the roots and lower part of the stem in dry spores and were transplanted to the rows in which the inoculated seed had been sown. The inoculated plants were washed free from all soil particles and the old attached seed washed and brushed until all traces of the spores were removed. They then were planted in the clean soil where the uninoculated seed had been sown. Thus, there was an exact reversal of position. Both sets also were inoculated, one on the original seed and the other when the plants were about two weeks old. The results are given in Table VIII.

TABLE VIII.—*Infection of three varieties of wheat by Urocystis tritici after inoculating seed and seedlings in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., from November 28 to December 13, 1921*

Variety.	Control.						Uninoculated seed; seedlings inoculated and transplanted to infested soil.			Inoculated seed; seedlings washed and transplanted to clean soil.		
	Clean seed in clean soil.			Inoculated seed in clean soil.								
	Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.		Total number plants.	Infected plants.	
		No.	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*		*No.*	*P. ct.*
Harvest Queen	16	0	0	18	14	77.8	30	1	3.3	34	30	88.2
White Federation	17	0	0	14	11	78.6	30	0	0	20	18	90.0
Little Club	17	0	0	14	14	100	35	1	2.9	32	30	93.8
All varieties	50	0	0	46	39	84.8	95	2	2.1	86	78	90.7

There was one plant each of Harvest Queen and Little Club infected in the lot which was inoculated when the plants were about two weeks old, but no infection appeared in White Federation. No other infection was noted on plants which were inoculated after the seedlings had emerged. There was 88.2 per cent or more of infection in each variety which was inoculated at the time the seeds were sown. From these results and from the data obtained in the previous experiment where

no infection occurred in 277 plants when inoculation was made after the coleoptile was broken, it would seem that in the greenhouse, at least, shoot infection was almost negligible.

RELATION OF CUTTING BACK INOCULATED PLANTS TO DEVELOPMENT OF SMUT

In addition to the possible infection of shoots during the process of tillering, there is another type of shoot infection which evidently comes from within the plant and is the result of the original seedling infection.

In the spring of 1921 inoculated plants of Bunyip, Cedar, and Comeback, grown in the greenhouse at Arlington Experiment Farm, apparently were free from the disease. Each plant had about four culms, all of which were yellow and well headed at the time of note taking. As these varieties had been practically free from the disease in the previous season, and as other infected varieties needed immediate attention, they were allowed to mature. However, the plants were watered each day, and small secondary shoots soon began to develop at the base of the mature culms. On later examination several of these shoots were found to be infected, even though the mature plants had shown no infection. The occurrence of the disease in these small, secondary shoots, while the headed culms of the same plants remained smut-free, possibly may be explained in two different ways. First, it may be assumed, as McAlpine has suggested, that they were infected by spores present in the soil at the time of emergence; or, second, that they were infected from mycelia already in the plant, which, for some reason, had not been able to form spores in the older leaf tissue.

Greenhouse experiments were conducted in 1921–22 to determine the possible source of infection in such secondary shoots and to study the effect of cutting back the plants at various stages of growth on the production of diseased secondary shoots. The results of the experiments in which seedlings had been inoculated immediately following rupture of the coleoptile and later, during tillering, have already been given. There was no infection of any shoot when the inoculations were made just after the coleoptile had been broken. Two plants out of a total of 95 were infected when inoculations were made after the seedlings were about two weeks old.

Again, 50 uninoculated seedlings of Harvest Queen were cut to the level of the ground immediately after the coleoptile became ruptured. The cut surface of each seedling and the soil around it were heavily inoculated with flag-smut spores. No infection developed in any of these plants. In later experiments no new cases of infection have been noted where inoculation of the soil and plants was delayed until the latter had emerged from the soil.

None of the soil in which the cut-back plants were grown was inoculated with flag-smut spores. The spores were sown with the seed and, therefore, were about 2 inches under ground. Thus there were no spores near the surface of the soil excepting those which might have been carried up through the soil by the plumule during its growth.

The crowns of wheat plants grown in the greenhouse were practically on the surface of the soil, so that often the secondary shoots never came in contact with the soil. The shoots arising from the base of the culms often were pressed closely to the culms. In cases of this kind the basal leaf sheaths of the culm incased both the secondary shoots and the lower

part of the culms until the shoots were from 1 to 3 inches high. The secondary shoots arose from the crown or from the first, second, and third nodes above the ground. Infection often was noted on shoots which arose from these points of origin. Plate 3, A, shows a cut-back plant with an infected shoot which arose from the second node, or the first node above ground, of a culm which had been cut. This infected shoot from the second node is enlarged in Plate 3, B.

From the fact that the infected shoots often arise from the nodes above ground, where the chances for infection are very few, and from the difficulty encountered in producing infection where inoculations were made after the plants were above ground, it would seem that the smut appearing on the secondary shoots arose from hyphae already inside the tissues of the host plant.

Seventeen winter varieties and 11 spring varieties were used in experiments to study the effect of cutting back wheat plants on the appearance of infection. These varieties were resistant, slightly susceptible, and, in a few instances, quite susceptible in previous varietal experiments. A susceptible spring and a susceptible winter variety, Bobs and Harvest Queen, were used as controls for infection. Four rows of each variety were sown 6 inches apart, placing 18 inoculated seeds 2 inches apart in each row. The plants in row 1 of each variety were cut back to about 1 inch in height when they had two leaves and were two to three weeks old. The plants of the second row of each variety were cut back to 2 to 3 inches in height when they had two or three small culms, or when infection was showing in the susceptible varieties used as controls. Plants in the third rows were cut back when beginning to head, and those in the fourth rows were left uncut and used as controls on the plants which were cut back. These different series of cut-back plants are hereafter referred to as rows 1, 2, 3, and 4.

The appearance of smut lesions was watched for closely, and all infected plants removed as soon as observed, so that in each instance only the smut-free plants were cut back. Of the 11 spring varieties treated in this manner 3 showed no infection and 2 an infection of 1.6 and 5.4 per cent, respectively, but which did not seem to be influenced by cutting back the plants. However, 5 varieties developed smut in the cut plants either earlier or in a greater percentage than in the plants which were not cut back. Of the 17 winter varieties, 10 showed no smut, while 4 showed only one or two infected plants. Thus in only 3 winter varieties was the development of smut influenced to any extent by cutting back the plants. The percentage of plants infected in each row of the 8 different varieties which became infected is given in Table IX.

There was no uniform plant response from each variety at each stage of development when cut back. In all varieties where the plants were cut back in the two-leaf stage, there was a greater percentage of infection than in the control rows which were not cut back. There was considerable variation in the percentage of infection in rows 2 and 3. Moreover, it was observed that clipping the culms not only influenced the percentage of infection, but, in many instances, the interval between sowing and appearance of smut was considerably shortened.

Clipping the plants after they had begun to head was the most favorable with regard to both the percentage and the appearance of infection in Bunyip. Small, secondary shoots at once began to develop in row 3, and the lesions appeared 17 days earlier in this row than in rows 2 and

4, and 34 days earlier than in row 1. Row 3 had a final infection percentage of 58.3 in comparison with 31.3 per cent in the control row. There was one plant in row 1 which was cut back twice before infection was noted—once in the two-leaf stage, and again after the plant had headed. Finally a small secondary infected shoot was put out from the first node above ground.

TABLE IX.—*Percentages of infected plants in eight wheat varieties inoculated with Urocystis tritici, and cut back at various stages of growth, in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1921–22*

Variety.	C. I. No.	Row number and stage when cut back.			Control.
		1 Two-leaf.	2 Time of normal infection.	3 Heading.	4 Not cut.
Bunyip	5012	40.0	40.0	58.3	31.3
Comeback	4991	90.9	72.7	84.6	[a] 20.0
Florence	5129	50.0	50.0	60.0	31.6
Sonora	4293	36.3	23.0	22.2	25.0
Pacific Bluestem	3019–3	83.3	43.7	42.8	33.3
Flint	6612	54.5	26.6	15.1	41.2
Fultz	3598	40.0	77.7	13.3	18.8
Jones Fife	5608	64.2	44.4	46.6	42.9

[a] Normal infection. An additional 73.3 per cent of the plants showed infection in small secondary shoots which appeared after the first culms had matured.

A striking difference in infection percentages was shown in the various rows of Comeback. It will be noticed from figure 1 that by the time there was 72.7 per cent of infection in row 1 there was 15.3 per cent in row 3, 9 per cent in row 2, and 20 per cent in row 4. None of the plants in rows 2, 3, and 4 had been cut back at this time. After all infected plants had been removed from the four rows the remaining sound plants in row 2 were cut the same day. Row 3 was cut back about one month later. Small shoots soon were produced at the crown and nodes, and many of these were infected with flag-smut sori. The dotted line in row 4, or the control, represents the infections which appeared in the small secondary shoots which were produced after the culms were practically mature.

Row 1, sown to Florence, produced 18.4 per cent more smut than the control, and the period of smut maturation was reduced by 30 days. Sixty per cent of the plants in row 3 were infected. This was the maximum for this variety, and exceeded by 28.4 per cent the infection recorded for the control row.

The final percentages of infection for Sonora did not show great differences. Row 1 produced 36.3 per cent, or 11.3 per cent more infection than developed in the control row. Row 2 contained 23.0 per cent of infection in comparison with 25.0 per cent in the control, but it should be noted that the total percentage of smut recorded for row 2 had developed 22 days before infection appeared in row 4, and 73 days before the last infected plant was noted in row 4. Although row 1 showed the highest percentage of infection, no lesions appeared in row 1 until 13 days after

they were first noted in the control row. This late appearance of infection in row 1 of Sonora was the same as in row 1 of Bunyip.

In Pacific Bluestem the control row showed 33.3 per cent of smut, while rows 1, 2, and 3 showed 83.3, 43.7, and 42.8 per cent, respectively. Moreover, the total 83.3 per cent of smutted plants in row 1 appeared 35 days before infection was first observed in the control row.

Row 1 of Flint showed 54.5 per cent of the plants infected in comparison with 41.2 per cent in the control and with 26.6 and 15.1 per cent in rows 2 and 3, respectively. The total 54.5 per cent of smutted plants in row 1 appeared 113 days before infection was first observed in the control row.

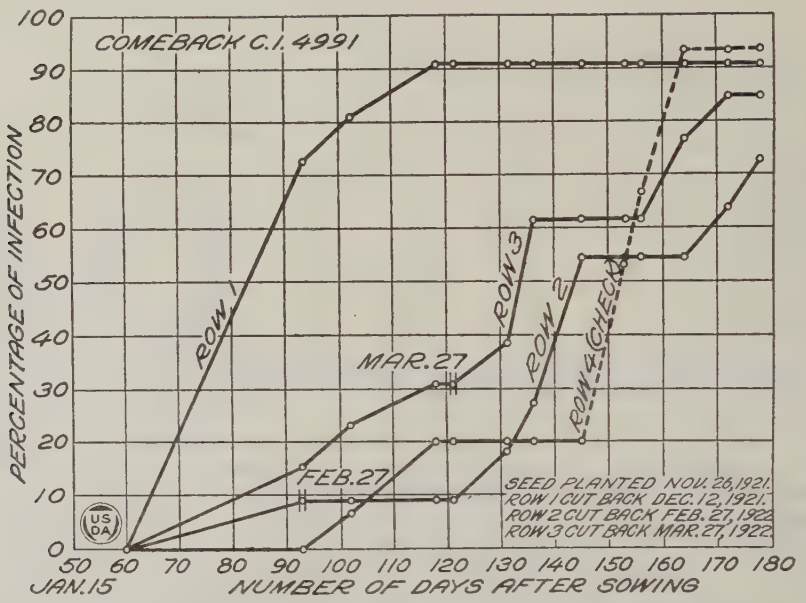

FIG. 1.—Graph showing the time of appearance of *Urocystis tritici* and the percentage of infection on plants of Comeback wheat cut back at different stages of growth while growing in the greenhouse at Rosslyn, Va., 1921–22.

Fultz showed 40 and 77.7 per cent, respectively, in rows 1 and 2 in comparison with 18.8 per cent in the control row, and the final infections were noted in the first two rows more than 30 days before smut was first observed in the control row. Smut lesions appeared about the same time in rows 1 and 2, but did not appear in rows 3 and 4 until over 30 days after their first appearance in rows 1 and 2.

In Jones Fife the sori appeared about the same time in the first three rows and over 30 days before they appeared in the control row. Row 1 had 21.3 per cent more smutted plants than the control row. Rows 2 and 3 showed only slightly higher percentages of infection than the control, though the appearance of the disease was noted in each row more than 30 days before it appeared in the control row.

It is significant to note that in many cases infection can be produced earlier and in greater percentage by cutting back the plants. As previously noted, the same effect was produced in Comeback by continued

watering after maturity. Row 4 of this variety, which had not been cut, showed only 20 per cent of the plants infected at the time the culms became yellow and were maturing. By continuous watering, small secondary shoots were produced, which probably were comparable to those produced when the plants were cut back. There was a total infection of 93.3 per cent of the plants, or an increase of 73.3 per cent, that was the result of the appearance of smut sori in these small secondary shoots. In view of these facts it is possible that many apparently quite resistant varieties are not suitable for breeding for resistance to flag smut. However, varieties showing this tendency to develop smut only in secondary shoots undoubtedly evidence a certain degree of resistance. Resistance was shown also by other varieties where the only infection on a whole plant consisted of a single very minute sorus. In one case a small lesion was found on a lower glume of a wheat head, thus indicating that the fungus had kept pace with the growth of the plant but was not able to sporulate extensively. In other instances there were only two or three minute sori on one leaf of an entire plant. This would indicate that the fungus was in the plant tissue even though the spores were not produced freely. As sporulation is the only superficial evidence of flag-smut infection, cytological investigation might determine the presence or absence of mycelia in the leaves and culms of plants which apparently are free from the disease. It also might indicate whether or not the mycelia generally passed beyond the lower nodes of the culms.

VARIETAL RESISTANCE

Experiments in varietal resistance were conducted during three winters, 1919, 1920, and 1921, in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. In addition to the commercial winter wheats grown in Illinois, several other varieties of spring and winter wheats were included. Twenty-one varieties were tested for three years, while others were tested for one or two years. All inoculations were made by placing the seed and a sufficient quantity of dry ground leaves containing spore material in an envelope and shaking until the seed was covered with spores. The inoculated seeds were sown 2 inches apart, and about 2 inches deep, in rows 6 inches apart in the greenhouse benches. In 1920 and 1921 small quantities of spore material also were placed on each inoculated seed before covering with soil. The soil was moist and in good tilth, and the air temperature was maintained approximately at 55°F. The data were obtained when the plants were beginning to head, or heading, except in cases of severe infection, which necessitated the removal of the plants before that time. Infection was noted for both culms and plants, except in 1922, when only the plant infections were recorded. The results for the three years are given in Table X.

Of the 21 varieties which were tested for three years only Fulcaster, Poole, Red May, Red Rock, Early Defiance, and Galgalos were free from flag-smut infection during that time. Of 17 additional varieties tested for two years Illini Chief, Treadwell, Turkey, and Peliss were free from infection. The highest percentages of smutted plants recorded for the most susceptible varieties in any one year were 78.9 per cent for Fultz, 93.1 for Harvest Queen, 86.7 for Jones Fife, 66.1 for Purplestraw, 72.9 for Red Wave, 98.4 for Bobs, 93.3 for Comeback, 92.7 for Cowra No. 3, 88.4 for Defiance, 98.2 for Hard Federation, and 100 per cent for Little Club and White Federation.

TABLE X.—*Infection of wheat, spelt, and emmer by Urocystis tritici produced by inoculating the seed with dry spores and sowing in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., during one or more of the three years, 1919, 1920, and 1921*

Crop and variety.	Source or C. I. No.	1919–20				1920–21				1921–22		Average number of infected plants.		
		Plants.		Culms.		Plants.		Culms.		Plants.				
		Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infect. ed.	Years averaged.	Plants.	Infected.
WINTER WHEAT.		*No.*	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*	*No.*	*P. ct.*		*No.*	*P. ct.*
China	180	.....	.....	.....	.....	50	2.0	293	0.3	9	11.1	2	2	3.4
Flint	6612	.....	.....	.....	.....	60	25.0	605	4.5	17	41.2	2	22	28.6
Fulcaster	6162	35	0	165	0	44	0	237	0	14	0	3	0	0
Fulcaster	Tenn.	.....	.....	.....	.....	52	11.5	269	3.7	15	0	2	6	9.0
Fultz	3598	40	45.0	364	31.3	57	78.9	340	54.4	16	18.8	3	66	58.4
Gipsy	5579	.....	.....	.....	.....	53	3.8	291	1.0	16	0	2	2	2.9
Goens	3428	.....	.....	.....	.....	59	6.8	366	1.1	17	5.9	2	5	6.6
Harvest Queen	5957	48	85.4	434	79.5	116	93.1	1,094	87.1	104	88.5	3	241	89.9
Do	Ill.	41	75.6	420	73.8	.....	.....	.....	.....	.....	.....	1	31	75.6
Honor	6161	.....	.....	.....	.....	52	0	290	0	8	12.5	2	1	1.7
Hussar	4843	.....	.....	.....	.....	.....	.....	.....	.....	46	0	1	0	0
Do	6553	.....	.....	.....	.....	.....	.....	.....	.....	29	0	1	0	0
Illini Chief	5406	.....	.....	.....	.....	58	0	348	0	14	0	2	0	0
Jones Fife	5608	.....	.....	.....	.....	45	86.7	284	75.7	14	42.9	2	45	76.3
Kanred	5146	37	5.4	217	.9	54	0	369	0	.....	.....	2	2	2.2
Martin	1974	.....	.....	.....	.....	61	9.8	350	2.0	12	0	2	6	8.2
Do	4463	.....	.....	.....	.....	.....	.....	.....	.....	60	0	1	0	0
Mealy	5824	45	2.2	196	1.0	53	17.0	273	.7	15	13.3	3	12	10.6
P–1066	5879	.....	.....	.....	.....	.....	.....	.....	.....	6	0	1	0	0
P–1068	5880	.....	.....	.....	.....	.....	.....	.....	.....	15	0	1	0	0
Poole	3489	46	0	189	0	56	0	313	0	14	0	3	0	0
Purplestraw	1915	.....	.....	.....	.....	62	66.1	425	42.6	.....	.....	1	41	66.1
Red May	5596	39	0	229	0	45	0	242	0	14	0	3	0	0
Red Rock	5976	43	0	222	0	50	0	267	0	13	0	3	0	0
Red Wave	5582	.....	.....	.....	.....	48	72.9	313	36.1	.....	.....	1	35	72.9
Treadwell	3527	.....	.....	.....	.....	45	0	419	0	10	0	2	0	0
Turkey	5966	.....	.....	.....	.....	58	0	485	0	17	0	2	0	0
SPRING WHEAT.														
Baart	1697	45	35.6	251	17.1	55	29.1	219	9.1	.....	.....	2	32	32.0
Bobs	4990	43	93.0	257	84.0	124	98.4	541	94.5	30	86.7	3	188	95.4
Bunyip	5012	49	2.0	158	.6	59	5.1	177	1.7	16	31.3	3	9	7.3
Cedar	4117	52	0	210	0	60	6.7	221	2.3	14	28.6	3	8	6.3
Chul	2227	.....	.....	.....	.....	62	0	258	0	14	14.3	2	2	2.6
Comeback	4991	48	0	211	0	64	43.8	223	14.8	15	93.3	3	42	33.1
Cowra No. 3	4119	41	92.7	264	89.8	47	87.2	370	81.4	31	0	3	79	66.4
Defiance	Calif.	43	88.4	317	52.7	61	24.6	242	9.5	.....	.....	2	53	51.0
Dicklow	3663	.....	.....	.....	.....	40	50.0	176	30.1	.....	.....	1	20	50.0
Early Defiance	6480	43	0	183	0	55	0	227	0	17	0	3	0	0
Florence	5129	44	0	157	0	46	13.0	187	3.7	19	31.6	3	12	11.0
Galgalos	2398	47	0	264	0	60	0	342	0	16	0	3	0	0
Hard Federation	4733	51	72.5	273	68.1	56	98.2	230	95.2	26	80.8	3	113	85.0
Little Club	4066	50	2.0	235	.9	56	100	324	95.7	45	97.8	3	101	66.9
Marquis	3641	51	7.8	292	2.4	63	3.2	270	1.1	15	0	3	6	4.7
Pacific Bluestem	3019–3	48	4.2	206	1.5	61	4.9	275	1.8	12	33.3	3	9	7.4
Peliss	1584	.....	.....	.....	.....	58	0	169	0	13	0	2	0	0
Propo	1970	51	35.3	247	16.6	64	62.5	322	51.9	.....	.....	2	58	50.4
Sonora	4293	51	2.0	185	.5	57	29.8	272	8.5	16	25.0	3	22	17.7
White Federation	4981	45	88.9	207	84.5	50	100	247	92.7	39	97.4	3	128	95.5
EMMER.														
Black Winter	2337	40	0	108	0	.....	.....	.....	.....	.....	.....	1	0	0
SPELT.														
Alstroum	1773	30	10.0	197	2.0	.....	.....	.....	.....	.....	.....	1	3	10.0

The percentage of infection for the same strains of many varieties was found to be much higher when grown in the greenhouse than when grown

in the field. Tisdale, Dungan, and Leighty (*15*) give the average percentage of infection for the different varieties grown in the field for a number of years. The same strains of some of the varieties grown by them in the field also were grown in the greenhouse by the writer. The average percentages of infected plants grown under both field and greenhouse conditions are given in Table XI.

TABLE XI.—*Percentage of infection of the same strains of wheat, spelt and emmer inoculated with Urocystis tritici and grown in the field at Granite City, Ill., and in the qreen house at Arlington Experiment Farm, Rosslyn, Va., in the same three years, 1919, 1920, and 1921*

Variety.	Source or C. I. No.	Field.		Greenhouse.	
		Average infection.	Number of years grown.	Average infection.	Number of years grown.
WHEAT.		*Per cent.*		*Per cent.*	
China	180	1.6	2	3.4	2
Dawson	6161	5.8	2	1.7	2
Fulcaster	6162	1.2	3	0	3
Fultz	3598	2.5	2	58.4	3
Gipsy	5579	.7	3	2.9	2
Goens	3428	2.5	2	6.6	2
Harvest Queen	5957	24.9	3	89.9	3
Do	Ill.	18.1	1	75.6	1
Hussar	4843	0	1	0	1
Illini Chief	5406	3.0	2	0	2
Jones Fife	5608	4.8	2	76.3	2
Kanred	5146	2.5	3	2.2	2
Martin	Wn. 1092	0	1	0	1
Mealy	5824	7.3	2	10.6	3
P–1066	5879	.3	2	0	1
P–1068	5880	0	2	0	1
Poole	3489	Trace.	3	0	3
Purplestraw	1915	9.7	2	66.1	1
Red May	5596	1.0	2	0	3
Red Rock	5976	.2	3	0	3
Red Wave	5582	Trace.	1	72.9	1
Treadwell	3527	2.8	3	0	2
SPELT.					
Alstroum	1773	0	2	10.0	1
EMMER.					
Black Winter	2337	0	2	0	1

Many of the varieties which were free from infection when grown in the greenhouse were reported as being slightly susceptible in the field. On the other hand, one of the most susceptible varieties, Harvest Queen (Red Cross), had a three-year average infection of 24.9 per cent in the field. The same strain of Harvest Queen grown in the greenhouse during the same three years had an average infection of 89.9 per cent, or a difference of 65 per cent. Other varieties which showed great differences in percentages of infection were Fultz, Jones Fife, Purplestraw, and Red Wave. The fact that the area quarantined for flag smut is located in the winter-wheat section has precluded any experiments on varietal resistance of spring wheat in the field.

SUMMARY

Flag smut of wheat occurs in the United States, Australia, Japan, China, India, South Africa, Italy, and Spain. Although heavy losses have been reported from other countries, it has been estimated that in the five years that flag smut has been known in the United States there has been less than 2 per cent of loss in the infested areas.

Flag smut of wheat is caused by *Urocystis tritici* Ķcke., which produces sori on the leaves, stalks, and glumes. Badly infected plants do not head. The spores do not germinate readily in tap or distilled water, and special conditions are necessary for uniform germination. Spores kept under laboratory conditions were viable for at least four years. They are able to live through the winter in the soil in the vicinity of St. Louis, Mo. These overwintered spores are capable not only of germinating but of infecting wheat plants.

Sowing inoculated wheat at successive dates in the fall at St. Louis, Mo., resulted in a general decrease in the percentages of infection. There was no infection in the sowing of November 14 or in later sowings.

Infection occurred at 6° to 12° C., 17° to 19°, and 21.5° to 23.5°, but did not occur at 25° to 26.6°. The highest percentage of infection occurred at 21.5° to 23.5°. The most favorable stage of growth for infection was in the seedling stage before the coleoptile was broken and before the seedlings emerged from the soil.

Infection occurred either earlier or in a greater percentage in a few varieties which had been inoculated and cut back at different stages of growth than in the same varieties which had not been cut back.

Fulcaster, Poole, Red May, Red Rock, Early Defiance, and Galgalos remained smut-free during the three years of this experiment; Illini Chief, Treadwell, Turkey, and Peliss, grown for only two years, also were smut-free. The percentages of infection of susceptible varieties grown in the greenhouse were considerably higher than those of the same strains grown in the field.

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PLATE 1

A.—Upper leaves of a wheat culm infected with *Urocystis tritici*, showing the long stripes, or sori.

B.—An enlarged portion of a wheat leaf infected with *Urocystis tritici*, showing the smut sori.

C.—Flag smut lesions on the lower glumes of an infected wheat head (*a*), and on the leaf sheath (*b*).

D.—Lesions of *Urocystis tritici* on the lower glumes (*a*), the rachis (*b*), and the upper part of the culm (*c*).

A
B
C
D
a
a
a
a
a
b
b
c
c

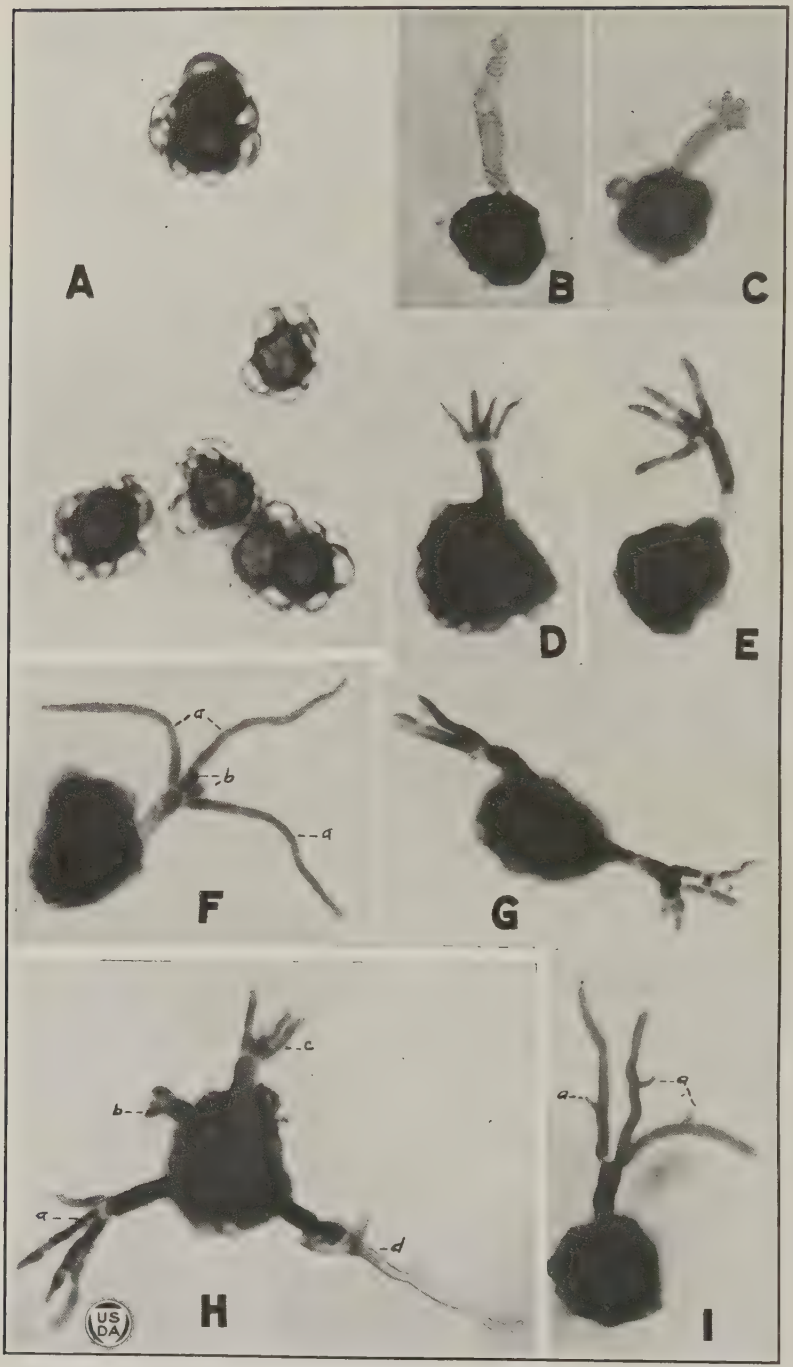
A
B
C
D
E
a
b
a
F
G
c
b
a
d
H
USDA
a
a
I

PLATE 2

Germination of spores of *Urocystis tritici*.

All figures were photographed with 4-mm. objective and No. 10 ocular by Miss Ruth Colvin, Office of Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture.

A.—Spore balls, each with one or two spores, invested by sterile cells.

B.—Germinating spore with promycelium.

C.—Germinating spore with sporidia just forming at apex of promycelium.

D–E.—Germinating spores with sporidia.

F.—Sporidia elongating, forming the so-called infection threads (*a*). Two sporidia (*b*) did not elongate.

G.—Spore ball with two spores germinating, each with sporidia at the apex of the promycelium.

H.—Spore ball with four spores in different stages of germination; (*a*) sporidia beginning to elongate, (*b*) sporidia just forming, (*c*) sporidia formed, and (*d*) sporidium elongated to form an infection thread.

I.—Three sporidia, each with a secondary sporidium.

PLATE 3

A.—A cut-back plant of Comeback wheat showing an infected shoot (*a*) arising from the second node of one of the culms.

B.—An enlargement of a portion of the secondary shoot (A*a*), showing the typical elongated smut sori.

A
B